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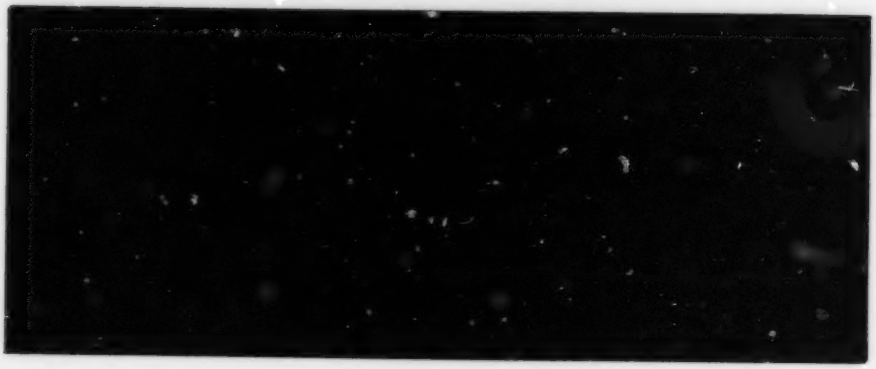

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Technical/Final Report
to
Saskatchewan Agriculture Development Fund

Alternative Feedstuffs For Saskatchewan Poultry

R-86-01-0061

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Final

Agriculture Development Fund

Project Title: Alternative feedstuffs for Saskatchewan poultry.

Applicant: David A. Christensen
Professor and Head
Dept. of Animal and Poultry Science
University of Saskatchewan

Co-Investigators: H.L. Classen
G.L. Campbell

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1. Abstract
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(a) Executive Summary

1. Abstract

The project represents part of an ongoing effort to improve feed grains for poultry by dietary enzyme supplementation to alleviate feeding problems caused by viscous carbohydrates occurring in barley, rye, and oats. More specifically the project was addressed to improve rye by enzymatic disruption of pentosans. Rye production is largely limited by market potential, which could be increased through greater use as a feed grain. A crude pentosanase preparation from Aspergillus niger, selected for enhanced pentosanase production, was fractionated into material of varying purity. All fractions improved chick growth, however, the pure pentosanase was less effective than the crude preparations indicating that a synergistic, coherent enzyme system may be necessary for optimal effect. The crude pentosanase preparations gave growth improvements approaching 100%.

2. Summary

Despite Saskatchewan being a major grain-producing region, much of our feed grain is not used extensively in poultry diets locally or world-wide. The latter has serious ramifications in marketing as the international poultry industry has a dominant role in the marketplace. Traditionally lack of use in this area has been attributed to the inherently higher fiber level found in barley, oats, or rye, which is incompatible with high energy poultry diets. A greater limitation are soluble fiber types that interfere with digestion and food passage. These may be simply and economically disrupted by adding appropriate enzymes to poultry diets. Past efforts have centered on β-glucanase-induced improvement of barley diets and this work is continuing.

The point this project addresses specifically is pentosanase improvement of broiler diets containing rye, which contains high soluble pentosan levels. Rye diets to which pentosanase had been added were vastly superior to rye control diets, although even with enzyme supplementation were not able to match a standard industry diet. Broiler chicks are desirable from a model standpoint due to their sensitivity; it is anticipated that the most logical end-use of rye diets with enzyme added would be in less sensitive areas, such as laying hens or swine.

Feeding pentosanase preparations of varying purity gives different broiler chick growth responses, which indicates that multi-enzyme systems are more effective than a single enzyme component. The separation of the active components provides a basis by which synergistic activity may be investigated. Although rye was examined specifically, pentosanase has also been suggested to play a role in enzymatic improvement of barley and oats, in conjunction with ß-glucanase, which is the principal enzyme involved in improving these grains.

A survey of extract viscosity of barleys grown in different locations across Western Canada indicated both genetic and environmental influence on this trait. Of particular concern is the relationship to climate - barleys ripening under moisture stress, as occurs over much of Saskatchewan, have elevated viscosity values. Noteworthy, too, was the observation that hulless barley ('Scout') was among the highest tested. When fed to broiler chicks high viscosity barleys were clearly inferior to low viscosity barleys, however, these differences were effectively eliminated with dietary enzyme supplementation. Although the response was smaller, low viscosity barleys also responded suggesting enzyme should be added to all diets containing barley.

Past successes in treatment of barley diets with an enzyme supplement has

Past successes in treatment of barley diets with an enzyme supplement has led to commercial application with several feed companies. Thus far, approximately 250,000 broilers have been fed barley diets with an enzyme supplement. Although the results have been successful, the cost spread between feed barley and wheat is presently low, which provides little financial incentive for producers to switch at this time. It is expected this will change in the future.

Interest in taking enzyme technology with Canadian barley into the international market has been expressed by the Canadian Wheat Board. To this end we are hosting two feed barley workshops (sponsored by the Canadian Wheat Board and the Canadian International Grains Institute) during May and April (1986). Confirmed participants are Korea, Japan, Malaysia, China, Israel, Bulgaria and the Philippines.

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#R-86-01-0061

UNIVERSITY OF SASKATCHEWAN
Saskatchewan Agriculture
"Alternative Feedstuffs for Saskatchewan Poultry"
Under the Direction of D.A. Christensen, Animal & Poultry Science
Statement of Receipts & Expenditures for the Period
July 16, 1986 - January 31, 1987

Receipts		
Saskatchewan Agriculture - September 1986		$ 35,000
Expenditures		
Salaries	$38,298	
Fringe Benefits	4,013	
Materials & Supplies	1,980	
Printing	55	
Telephone	433	
Feed	1,806	
Travel	230	46,815
Overexpenditure as of January 31, 1987		(11,815)

PROJECT DIRECTOR

(Ms.) C.M. BOYER
RESEARCH ACCOUNTANT

K-86-01-0061

Technical Report I

IMPROVEMENT OF THE NUTRITIONAL VALUE OF RYE DIETS FOR FOR BROILER CHICKENS BY SUPPLEMENTATION WITH PURIFIED PENTOSANASE (ARABINOXYLANASE)

ABSTRACT

1. A pentosanase component of a crude enzyme product was concentrated and added to chick diets. Four levels of purity were examined: a) the crude enzyme product, b) a crude water extract, c) a pentosanase purified using ion-exchange chromatography and d) residual pentosanase eluted with salt from the ion-exchange column.

2. All preparations exhibited high viscosity-reduction capability when tested against a soluble pentosan preparation isolated from rye grain. The purified pentosanase contained virtually no activity against carboxymethyl cellulose, pectin, starch or protein. Purity was verified using polyacrylamide gel electrophoresis, which indicated a single predominant protein.

3. The absence of arabinose release during rapid viscosity reduction of pentosan indicated that the purified pentosanase was an endoarabinoxylanase 1,4-β-D-xylan xylanohydrolase, EC 3.2.1.8) without arabinosidase activity.

4. When added to broiler chick diets containing rye as the only cereal, all enzyme preparation significantly improved growth and feed conversion. The results corroborated that soluble pentosans in rye act as growth depressants. At equivalent viscosity reduction activity the purified pentosanase was less effective than other preparations, suggesting that multiple enzymes were necessary to elicit maximum benefit.

5. Alternatively, since the purified enzyme was less pH stable it might have lost activity in the acidic environment of the foregut. It possessed greater temperature stability than the crude and salt-eluted pentosanase.

BACKGROUND AND OBJECTIVE

Broiler chicks given rye as a cereal source usually fail to thrive, which has been attributed to highly viscous pentosans (arabinoxylans) that reduce digestibility (Antoniou et al., 1981, Campbell et al., 1983; Ward and Marquardt, 1983) and retard food passage (McKay, 1980). These effects may be relieved by water extraction (Fernandez et al., 1973), soaking (Fry et al. 1958), or destruction of the pentosans by gamma irradiation (Campbell et al., 1983; Patel et al., 1980). An approach with greater practical applicability would be to supplement rye-containing diets with enzymes capable of degrading pentosan within the gut lumen. ß-glucanase supplementation of chick diets containing barley disrupts the viscous condition induced by solubilized endospermal ß-glucan (Classen et al. 1985; Edney et al., 1987; Hesselman et al., 1982), and increases growth. Similar efforts to improve rye diets for chicks (Bores et al., Fengler and Marquardt, 1985; Patel and McGinnis, 1980) have employed enzyme sources in which pentosanases were not the principal active component. Alleviation of growth depression with specific enzymes could provide a non-disruptive means of quantitatively determining the contribution of intact pentosans to growth depression of chicks fed rye, as well as providing a potential means by which rye may find greater use as a poultry feed. Also, proof that the primary deleterious component of rye are in fact pentosans has been hampered by extraction and purification difficulties, which are necessarily destructive to some extent.

The pentosanase component of an Aspergillus niger enzyme product (GNC

Bioferm Inc., Saskatoon, Saskatchewan) was concentrated and added to broiler chicken diets. Four enzyme preparations were used: the crude enzyme product, a crude enzyme extract, a pentosanase purified using ion-exchange chromatography and residual enzymes (including pentosanase) eluted with salt from the ion-exchange column.

MATERIALS AND METHODS

Enzyme fractionation

The crude enzyme product (250 g) was extracted with deionized water (3L), concentrated and dialyzed against water (1L) and 0.005 M sodium phosphate buffer (pH 6.8) (1L), respectively, using an ultra filtration unit (Amicon model DC2, Amicon Canada, Oakville, ON) equipped with a 1 ft^2 hollow fibre cartridge (10,000 M.W. cut-off). The concentrate was freeze-dried to yield the crude enzyme extract. Pentosanase was purified using DEAE-Sephacel (Pharmacia, (Canada) Ltd., Dorval, Quebec) chromatography, which was carried out using a column of 6.5 cm diameter and a bed volume of 950 ml. Aliquots of ultra-filtered crude extracts (300 ml) were applied to the column, and pentosanase activity was partially eluted with the above phosphate buffer, yielding the purified pentosanase. The remaining activity was eluted with the same buffer containing 0.4 M NaCl, yielding the salt-eluted pentosanase. The elution rate was 100 ml/h, and fractions of 25 ml were collected. Pooled fractions were concentrated by ultrafiltration, the NaCl dialyzed out against deionized water, and the retentate freeze-dried. Ultra-filtration and chromatography were carried out at $4^{o}C$.

Enzyme fractions were examined for constituent proteins using polyacrylamide gel electrophoresis on 8-25% non-denaturing gradient gels (Phast System, Pharmacia). Proteins were stained with Coomassie blue.

Pentosan substrate preparation

Intact rye grain was hammermilled (1/32" screen), and the resulting rye flour (1 kg) stirred into 15 L NaOH (0.1N). Following soaking at room temperature for 24 hr, the mixture was centrifuged (using a semi-continuous steam-driven centrifuge (50,000 RPM, Model TIP Pressurtite, Penwalt-Sharples, Warminster, PA). The pH of the supernatant was adjusted to 7.5 with glacial acetic acid and then digested with porcine pancreatin (14 g, Grade VI, Sigma Chemical Co., St. Louis, MO) at 32°C for 24 h, and recentrifuged (Sharples, 50,000 RPM). Three volumes ethanol (95%) were added to the supernatant and the mixture allowed to stand overnight (4°C). The precipitated pentosan was collected by filtration, washed sequentially with ethanol (95%), acetone, and ethyl ether, and dried under vacuum (30°C).

The pentosan isolate was hydrolyzed and derivatized (Blakeney et al., 1983), and analyzed for component sugars by gas-liquid chromatography (GLC, Sigma 2000 Gas Chromatograph, Perkin Elmer Corp., Norwalk, CT) using a capillary column (Durabond DB1701, 25 µm 30 m, Terochem Laboratories Ltd., Edmonton, AB). Column temperature program was 200 to 220°C at 4° per min, with a five min hold-time at 220°C. Deoxyglucose was used as an internal standard. Other analyses used to establish pentosan purity were protein (Lowry et al., 1951) and total reducing sugars (Miller, 1959).

Enzyme Analyses

Pentosanase, ß-glucanase, carboxymethylcellulase (CMC'ase), -amylase, and pectinase activities were assayed by a viscometric method. Six ml of substrate solution; 0.5% pentosan (prepared in-house, see above), 0.5% ß-glucan (barley, Sigma), 1.25% carboxmethylcellulose (Sigma , 2.0% starch (boiled, soluble potato starch, Sigma) or 0.5% pectin (citrous, Sigma) in 0.1

M sodium acetate buffer (pH 5.0) were incubated at 30°C in a kinematic tube viscometer (Cannon-Fenske type, model 15-731, Fisher Scientific, Edmonton, AB). After temperature equilibration, 0.1 ml of enzyme solution (diluted appropriately) was added and 4 to 6 flow times determined over a 15 min period. Flow rates were transformed according to the formula $1/n_{sp} = \frac{1/t_s - t_b}{t_b}$ where n_{sp} is the specific viscosity coefficient, t_s is the flow time of the substrate/enzyme reaction mixture, and t_b is the flow time of the buffer. Plotting of $1/n_{sp}$ (dimensionless) versus reaction time gave a straight line, the slope of which represented enzyme activity. Thus, one unit (U) of activity was defined as a change of one $1/n_{sp}$ min^{-1}. The viscometric assay was linear up to at least 0.03 U ml^{-1} of reaction mixture.

Protease activity measurement was based on the release of dye from azocasein. Enzyme solution (10 ml) was added to 1% azocasein (10 ml, Sigma) and incubated at 30°C. Samples were withdrawn periodically over 30 min, mixed immediately with 10% trichloracetic acid (2 ml), centrifuged, and absorbance of the supernatant read at 335 nm. One unit of protease activity was defined as a change of one OD unit min^{-1}.

Arabinosidase activity was determined by GLC of pentosan hydrolysates. Enzyme solution (0.1 ml) was added to 0.5% pentosan in 0.1 M acetate buffer (pH5, 9.9 ml) and incubated at 30°C. Samples were withdrawn at timed intervals, inactivated by heating in a boiling water bath (5 min), and quantitated by GLC as described earlier. One unit of arabinosidase activity was defined as one umole arabinose released per min. The same pentosan hydrolysates were monitored for total reducing sugar release with the dinitrosalicylic acid method, using xylose (Sigma) as a standard.

Enzyme Stability

Stability of pentosanase as a function of pH was determined as follows: freeze-dried enzyme preparations were dissolved in deionized water at 135 U ml^{-1}. Just prior to incubation at 40°C, the solutions were diluted 10 fold with 0.1 M sodium acetate - HCl of varying pH between 2 and 7. After 30 min incubation, the remaining pentosanase activity was stabilized by a further 10 fold dilution with 0.1 M sodium acetate buffer (pH 5.0) and assayed by the viscometric method.

Temperature stability of pentosanase was determined by dissolving freeze-dried enzyme preparations (135 U ml^{-1}) in 0.1 M sodium acetate. The solutions were incubated in a water bath at varying temperatures between 40 and 70°C for 15 min. After rapid cooling, and dilution (100 fold) with above acetate buffer, the remaining pentosanase activity was assayed.

Chicks, management and diets

Growth studies were conducted using commercial broiler chicks housed in battery brooders. Four pens were used per dietary treatment, each containing six male chicks assigned randomly. The test duration was 0-3 wk. The basal diets contained (g/kg) rye ('Cougar' or 'Musketeer',581 g), soybean meal (300 g), tallow (80 g), limestone (15 g), dicalcium phosphate (17 g), iodized salt (2.5 g), DL methionine (1.5 g) and vitamin-mineral premix (3g, Edney *et al.*, 1986). The enzyme preparations were added to the complete diet. The test diets were given as mash, and both feed and water were provided *ad libidum*.

arabinose and xylose release, and total reducing sugars (xylose-equivalent) are shown in Figure 3. Viscosity declined rapidly with little free sugar release (arabinose, xylose, or total reducing sugars). The enzyme preparations did exhibit pronounced differences in free sugar release with prolonged incubation. The salt-eluted pentosanase effected the greatest arabinose and xylose release, and greatest overall breakdown of the pentosan as shown by final reducing sugar levels. Free arabinose and xylose were negligible in purified pentosanse incubations, and intermediate in the case of the crude pentosanase extract.

Pentosanase stability

Exposure to temperature and pH extremes indicated varying stability of the pentosanase component of each fraction (Fig. 3). The purified preparation was more temperature-stable than the salt-eluted and crude extract no activity loss was observed below 60°C, after which activity dropped precipitously, reaching 0 at 70°C. In contrast, the salt-eluted pentosanase was only stable to 40°C, and the crude extract was stable to 45°C, reflecting the fact that the latter was a mixture of pentosanase components. Conversely, the purified pentosanase exhibited greater sensitivity to a low pH than the other two fractions (Fig. 4); activity dropped rapidly below pH 4. The salt-eluted pentosanase was the most pH stable, retaining 70% activity following exposure to pH 2. Again the crude extract showed intermediate stability.

Chick bioassay

Chicks given rye supplemented with enzyme preparations to provide uniform levels of pentosanase activity (30,000 U/Kg) were heavier at 3 wk than the control chicks ($P<.05$, Table 2). There were differences among enzyme

preparations; chicks fed purified pentosanase were lighter than those given the crude extract or salt-eluted pentosanase ($P<.05$). Chicks given crude extract were lighter ($P>.05$) than those given salt-eluted pentosanase. Feed/gain followed the same directions as that described for body weight, although the differences were not always statistically significant.

The dose-response relationship between enzyme level and chick performance was examined for the crude enzyme product and the crude extract (Table 3). Both preparations yielded significantly higher body weights ($P<.01$) and lower feed/gain ($P<.01$). Body weight increased incrementally with higher enzyme levels to a maximum of 483 g when chicks were given 120,000 U/Kg crude extract, with a corresponding feed/gain of 1.69.

The response to increasing levels of crude enzyme product was also examined in a second rye sample (Musketeer, Table 3). Chicks fed Musketeer rye with crude enzyme product were heavier ($P<.01$) and converted feed more efficiently ($P<.01$).

DISCUSSION

Previous efforts to improve rye as a poultry feed by enzyme supplementation have met with mixed results. Addition of a pectinase enzyme to rye diets fed to chicks has improved gain and feed conversion in broiler chicks (Patel *et al.*, 1980; Grammar *et al.*, 1982); and the same enzyme partly restored depressed egg production in laying hens given rye (Patel and McGinnis, 1980), although this effect was transitory and production declined to control levels by eight weeks. These authors discount the importance of viscosity reduction in the improvement noted, although Grammar *et al* (1982) did observe that the 'pectinase' reduced viscosity of a rye water-extract, which is indicative of

contaminating pentosanase activity.

Various cellulase preparations are also likely to contain hemicellulolytic activity with the desired affect; a crude cellulase (Trichoderma viride) with viscosity reduction capability improved growth, feed conversion, and digestibility for chicks given rye (Fengler, 1986; Marquardt and Fengler, 1985). Broz and Frigg (1986) reported success with one cellulase, but not another, which again points to cellulase itself as not responsible for the noted improvement. Boros et al. (1985) added an enzyme preparation with xylanolytic activity to chick diets, and observed no growth response; although the same material did improve rye when a wet incubation process was employed.

Our results demonstrate that an appropriate enzyme material may improve growth and feed conversion of chicks fed rye. For both rye varieties (Cougar and Musketeer), chick growth and feed conversion were curvilinear functions of enzyme level, with the majority of benefit occurring at initial levels. Purification of the pentosanase component of the crude material resulted in an enzyme preparation practically devoid of contaminating enzyme activity. Chick performance was improved significantly when purified pentosanase was included in the rye diet, however, it was still well below that attainable with either the crude extract or salt-eluted pentosanase, despite the same enzyme level being added in terms of pentosan viscosity reduction.

One possible explanation for the poorer response to the purified pentosanase is that a single enzyme was not as effective as a multiple enzyme system. Endo-hydrolysis of pentosans may require adjacent unbranched xylose residues (Aspinell, 1970), leading to the conclusion that optimal degradation of the highly substituted pentosans may be facilitated by arabinosidase activity. The delayed appearance of free xylose in vitro supported this, however, the ability of all enzyme preparations to effectively lower the

viscosity of pentosan solution *in vitro*, with limited free sugar release points to a single enzyme (endo-arabinoxylanase) as responsible for viscosity reduction. This could also reflect sufficient unsubstituted regions within the isolated pentosan for attack by a single enzyme, which in turn may not be completely representative of the native pentosan occurring in intact grain. The arabinosidase activity of the enzyme preparations did correspond to the magnitude of improvement in the chick bioassay.

The presence of other enzyme systems may facilitate degradation by solubilizing insoluble pentosan, thus increasing its susceptibility to enzyme attack. Kulp (1968), studying pentosans in wheat and their role in bread-making, observed that different enzyme systems with similar activity on soluble pentosans varied in ability to solubilize, and suggested that a synergistic attack of pentosanases and cellulases may be required for solubilization of the pentosan-cellulose complex.

The growth response of chicks to several pentosanase preparations demonstrated unequivocally that pentosans occurring in rye are growth depressing. It was not possible to distinguish whether the variable effectiveness of the pentosanase preparations was due to other enzymes, or it was simply a matter of differential enzyme stability. The purified pentosanase was rather unstable below pH 4 which would likely result in variable inactivation in the acidic environment of the foregut of the chick. The purified enzyme was substantially more temperature stable than the other preparations, which would be desirable in practical applications where diets are normally steam-pelleted. Pentosan destruction within the gut lumen must be accomplished in a harsh environment; it may be anticipated that numerous factors in the intestinal mileu may differentially affect pentosanase activity.

CONCLUSIONS AND IMPACT

All pentosanase enzyme preparations improved growth and feed conversion of broiler chicks given rye. The response of chicks given different preparations with the same pentosanase activity varied, suggesting multiple enzymes were necessary to elicit maximum response. Alternatively, differing enzyme stability to the acidic environment of the foregut may differentially affect enzyme activity. The enzyme quantity required to elicit the maximum response was far greater than that which could be economically applied. Lesser enzyme quantities, however, may well suffice in less sensitive classes of livestock such as laying hens or swine. It may also be possible to increase enzyme efficiency with enzyme combinations.

The response of chicks fed rye to dietary enzyme provide a promising indication that rye may, with enzyme addition, find a more prominent position in livestock feeds.

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Table 1. Composition of enzyme preparations used in rye diets

Preparation	Pentosanase	ß-Glucanase	CMC'ase	α-amylase	pectinase	protease	arabinosidase[3]
Crude Extract	30,000[1] (150)[2]	17,200(86)	640(3.2)	18(0.09)	106(0.53)	1.63(0.032)	280(1.4)
Purified	30,000 (997)	690(23)	12(0.41)	0(0)	3.6(0.12)	0(0)	0(0)
Salt-eluted	30,000 (102)	44,394(151)	735(2.5)	27(0.09)	61(0.21)	0.71(0.010)	735(2.5)

1 U/kg diet.

2 ( ) U/mg protein.

3 From Fig. 3B.

Table 2. Response of broiler chicks fed rye diets (Cougar) with common pentosanase levels from different preparation procedures

Pentosanase Preparation	Body weight (g)	Feed/gain
0 (control)	289[a]	2.11[b]
Crude extract	440[c]	1.76[a]
Salt-eluted	468[c]	1.81[a]
Purified	352[b]	1.96[ab]
SE	15	.05

[1] 30,000 U/kg provided for all enzyme preparations.

abcd means with different superscripts are significantly different ($P<.05$, SNK test).

Table 3. Body weight and feed/gain of broiler chicks fed rye diets (Cougar or Musketeer) with varying levels of crude enzyme product (CP) or crude extract (CE).

	Cougar rye			
	Body weight (g)		Feed/gain	
Level[1]	CP[2]	CE[2]	CP[2]	CE[2]
0 (Control)	289		2.11	
15,000 U/kg	398	415	1.79	1.88
30,000 U/kg	402	440	1.84	1.76
60,000 U/kg	462	474	1.78	1.71
120,000 U/kg	-	483	-	1.69
SE	15		.06	
	(P<.01)[2]	(P<.01)	(P<.01)	(P<.01)
	Musketeer rye			
0(control)	242		2.05	
3200 U/kg	287		1.92	
32000 U/kg	386		1.70	
160,000 U/kg	434		1.68	
320,000 U/kg	380		1.76	
SE	19		.05	
	(P<.01)		(P<.01)	

[1] pentosanase activity.

[2] Level of significance refers to polynomial regression analysis (quadratic).

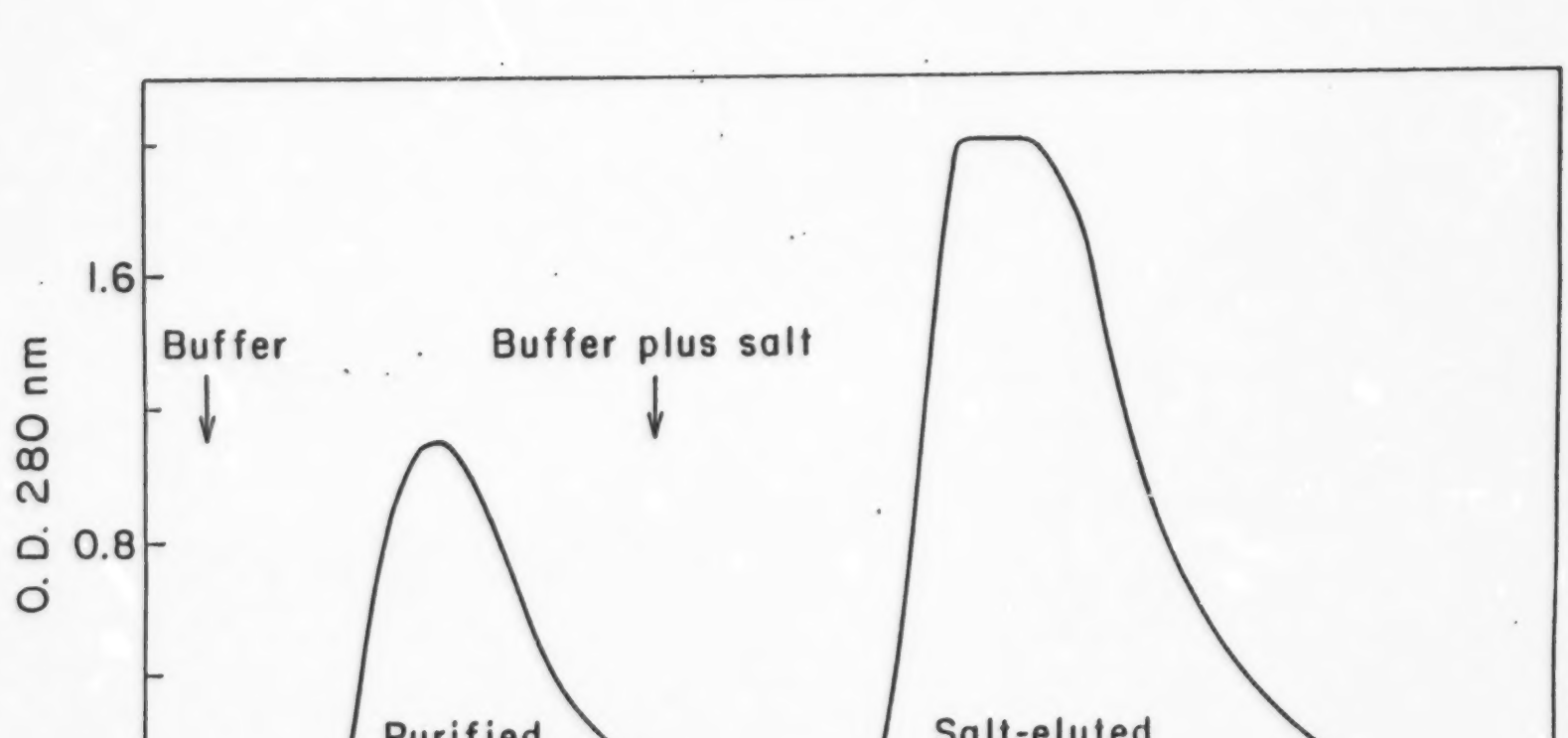
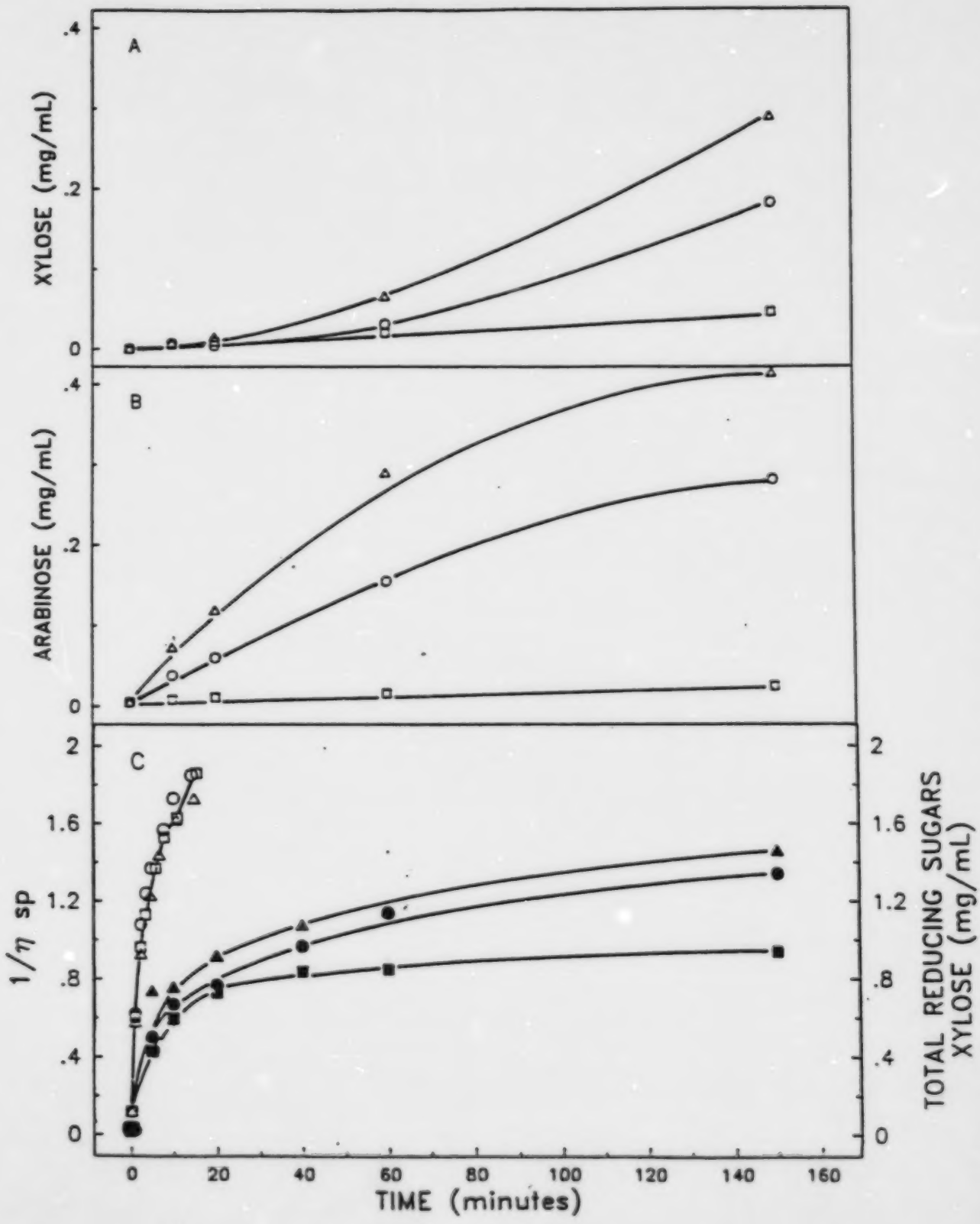
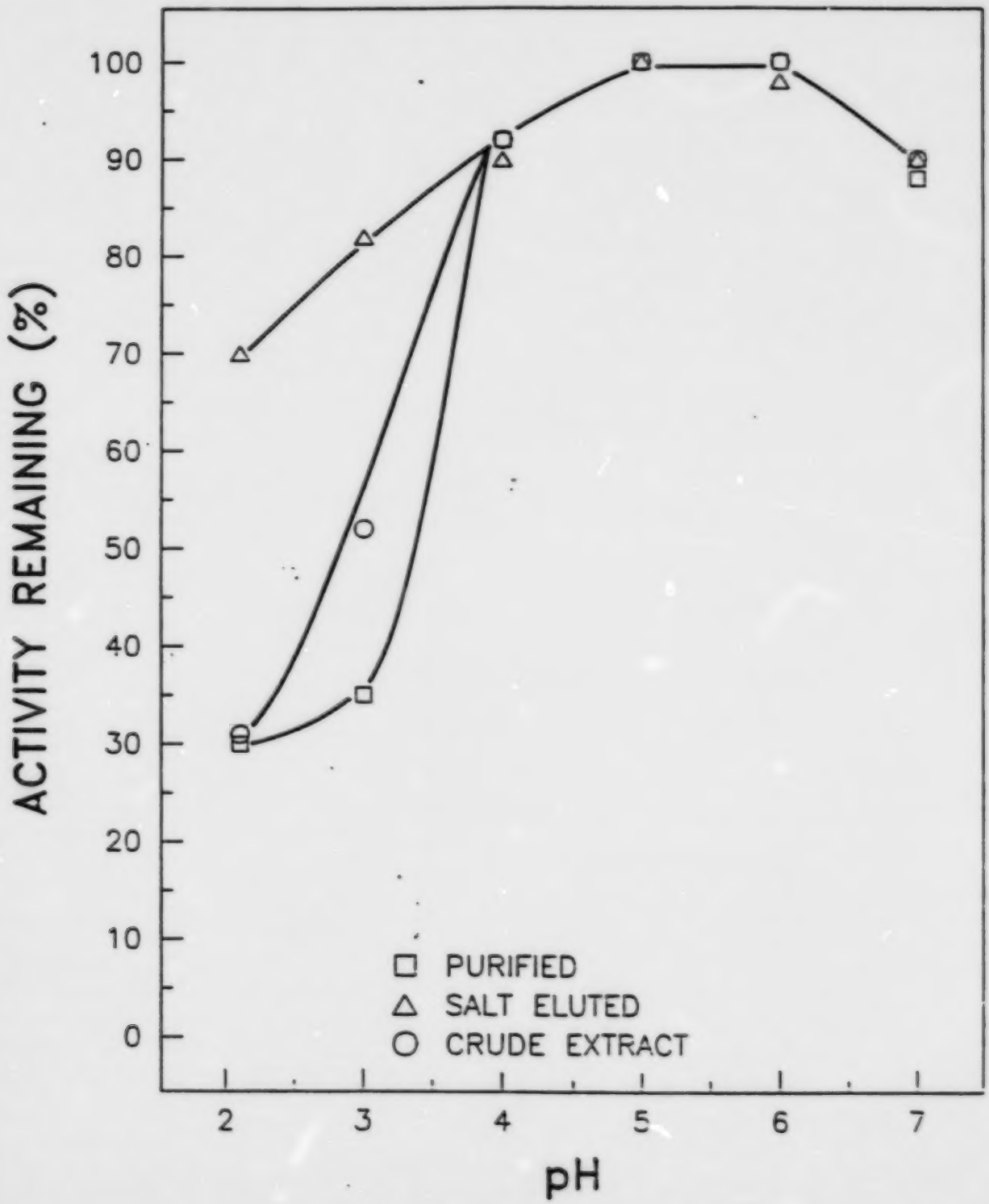
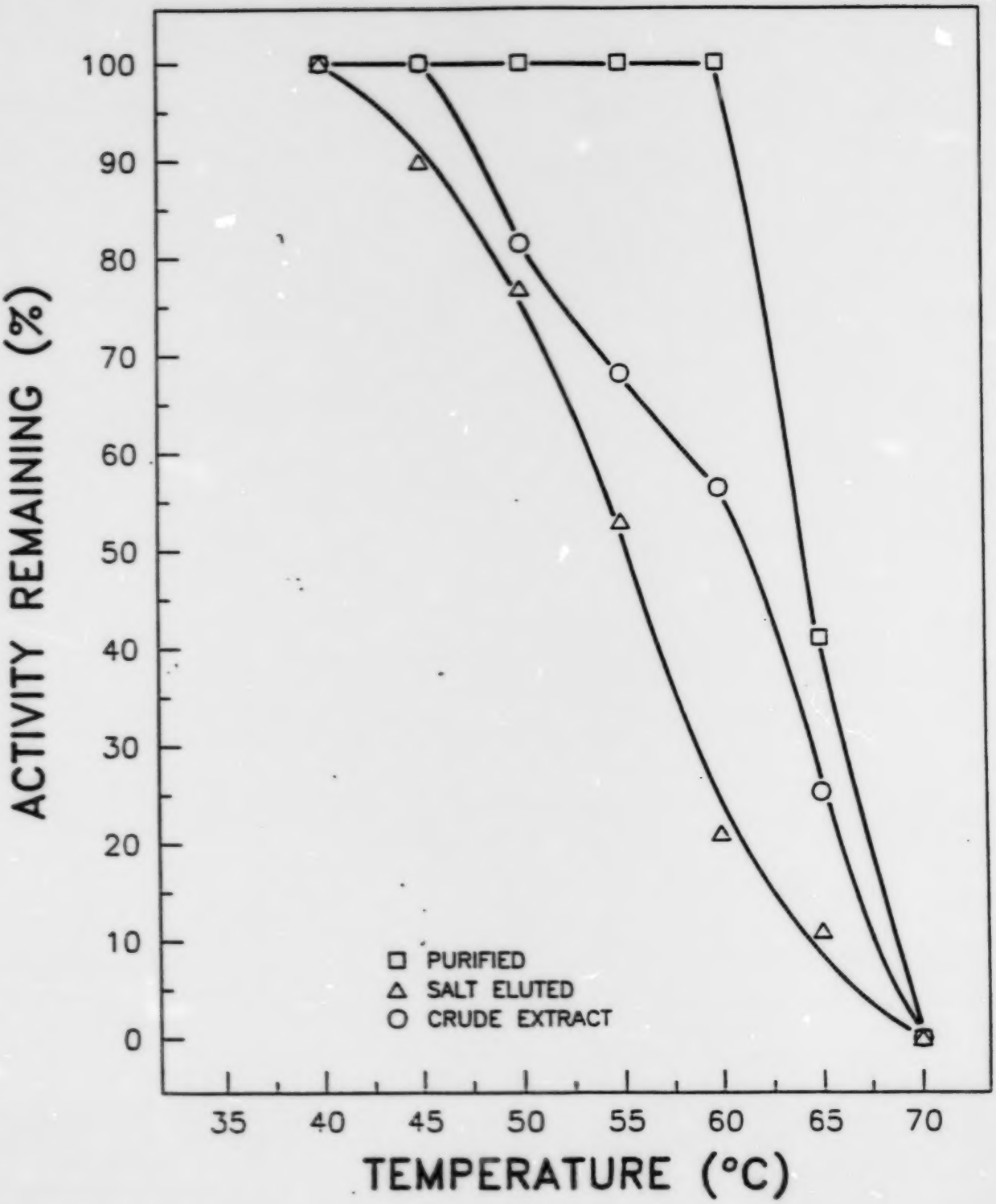

1.6
0.8
0
O. D. 280 nm
Buffer
Buffer plus salt
Purified
Pentosanase
Salt-eluted
Pentosanase
0
32
64
96
128
160
FRACTION NUMBER

Fig. 2 (Plate not available at time of preparation).


A
XYLOSE (mg/mL)
.4
.2
0
B
ARABINOSE (mg/mL)
.4
.2
0
C
1/η sp
2
1.6
1.2
.8
.4
0
TOTAL REDUCING SUGARS
XYLOSE (mg/mL)
2
1.6
1.2
.8
.4
0
0
20
40
60
80
100
120
140
160
TIME (minutes)


ACTIVITY REMAINING (%)
100
90
80
70
60
50
40
30
20
10
0
2
3
4
5
6
7
pH
PURIFIED
SALT ELUTED
CRUDE EXTRACT


ACTIVITY REMAINING (%)
100
90
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PURIFIED
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CRUDE EXTRACT

A-86-01

Technical Report II

CULTIVAR AND ENVIRONMENTAL DIFFERENCES IN EXTRACT VISCOSITY OF BARLEY AND ITS EFFECT ON THE NUTRITIVE VALUE FOR BROILER CHICKENS

ABSTRACT

Sixteen barley cultivars selected for variation in extract viscosity were grown at five diverse locations in Western Canada. Genotype and environment significantly affected extract viscosity, endogenous ß-glucanase, and protein levels. Differences in extract viscosity among locations were most apparent for high viscosity barleys; those exhibiting low viscosity were more uniform across locations. Low viscosity barleys tended to contain more endogenous ß-glucanase than the high viscosity types, and no clear relationship existed between protein level and extract viscosity.

Eight barleys representing high (Minerva, Voda, Lami, and Yugoslavian) and low (Triumph, Nirasaki, Klages, and Riso) viscosity traits were fed to broiler chickens in a three week growth study. Chicks fed low viscosity barleys gained significantly faster ($P<.01$), and converted feed more efficiently ($P<.01$) than those given high viscosity barley. Two high (Minerva and Yugoslavian) and two low (Triumph and Nirasaki) viscosity barleys, grown a second crop year, were fed to chicks with or without enzyme supplementation (ß-glucanase, pentosanse, Aspergillus niger). Chicks given low viscosity barleys with no enzyme addition surpassed their counterpart given high viscosity barleys with respect to growth ($P<.01$) and feed conversion ($P<.01$). Enzyme supplementation improved chick performance in both barley types, however, the response magnitude was greater for high viscosity barleys.

BACKGROUND AND OBJECTIVES

A major factor detracting from the nutritional value of barley for broiler chickens is ß-glucan originating from endospermal cell walls (Burnett, 1966; Gohl et al., 1978; Hesselman et al., 1982; White et al., 1982). Solubilized ß-glucan and the resulting viscous condition within the chick intestine interferes with nutrient assimilation (Campbell et al., 1986; Classen et al., 1985; Edney et al., 1987) and food passage (Gohl and Gohl, 1977). It is also suggested that insoluble ß-glucan impedes accessibility of starch granules to amylolytic activity within the intestine (Hesselman and Aman, 1986), which may not be conceptually distinct from the viscosity model if endospermal cell walls undergo partial solubilization during digestion. Relief from these impediments on digestion is most conveniently accomplished with dietary ß-glucanase supplementation, which imparts sufficient damage to eliminate its viscosity characteristics (White et al., 1983). ß-glucanase, possibly in conjunction with pentosanase, also disrupts the architecture of the endosperm cell wall (de Silva et al., 1983, Hesselman and Aman, 1985).

ß-glucan in barley varies according to sample—genotype, environment, and maturity at harvest are known to play a role (Lance, 1984; Hesselman and Thomke, 1982). Moisture stress brought on by drought conditions during kernel filling elevates both acid soluble and total ß-glucan deposition (Aastrap, 1979). Corresponding regional differences have been noted for barley as a poultry feed; barley grown in western U.S. are considered inferior to those from eastern regions (Burnett, 1966; Willingham et al., 1960).

Similar differences in feeding value of barley for broiler chickens may exist in barleys grown in western Canada, as a consequence of variable ß-glucan content. If barley is to gain a stronger position in poultry diets, it

is desirable to ascertain the potential impact of lowering ß-glucan genetically, or implementing a screening system by which barleys are selected by lot. An alternative would be to supplement diets with an enzyme supplement, however, it is unclear whether selected diets should be supplemented, or the enzyme should be added indiscriminately to all barley diets.

Sixteen barley cultivars representing high or low extract viscosity traits were selected from an initial screening of sixty barley cultivars, examined for extract viscosity and to ß-glucan content (Rossnagel and Ballance, unpublished data). The sixteen selected barley cultivars were subsequently grown at five diverse locations in Western Canada; Winnipeg, Brandon, Saskatoon, Lethbridge, and Beaverlodge, for two successive crop years. All barley samples were examined for acid buffer - extract viscosity, endogenous ß-glucanase, as well as total protein. Eight barley samples, four each high (Minerva, Yugoslavian, Wong, and Lami) and low viscosity types (Riso, Klages, Triumph, and Nirasaki) were fed to broiler chickens to ascertain the impact of viscosity on chick growth and feed conversion. A second experiment studied two high (Minerva and Yugoslavian) and two low (Triumph and Nirasaki) barleys with and without enzyme supplementation, to determine the interaction between barley type and enzyme supplementation. The principal components of the enzyme supplement (Grootwassink et al., 1987) were ß-glucanase and pentosanase.

MATERIALS AND METHODS

Trial management

Two trials were conducted using day-old commercial broiler cockerels housed

in heated battery brooders. Four pens each containing six chicks were assigned to each dietary treatment. Both the test diets, fed as dry mash, and water were supplied ad lib for the duration of the three week trial period. The test diets contained barley (582 g/kg), soybean meal (300 g/kg), tallow (80 g/kg), iodized salt (25 g/kg), DL-methionine (15 g/kg), and vitamin-mineral premix (2 g/kg, Edney et al., 1987). The enzyme supplement was added at 0.25 g/kg where indicated.

Viscosity determination

One g barley (1 mm screen, Udy Cyclone Mill) was mixed with 20 ml buffer (82.8 ml HCl and 7.46 g KCl to one l, pH 1.5) maintained at 40°. The samples were incubated 15 min. at 40°, with mixing at 5 min intervals, and centrifuged (10,000 g). The supernatant was filtered through cheese cloth to remove coarse particles, and 5 ml pipetted into a cannon-fenske viscometer (Fisher Scientific Ltd., Edmonton), also maintained at 40°. Extract viscosity was expressed as Nrel=ts/to, where ts is the flow time of test solutions and to is the flow time of distilled water.

Endogenous ß-Glucanase determination

ß-glucanase was determined using a radial gel diffusion technique (Edney et al., 1986; Martin and Bamforth, 1983) in which barley ß-glucan (US Biochemical Corp., Cleveland, OH) is incorporated in an agarose gel and activity related to enzymatic clearing surrounding wells in which extracts are placed. The intact ß-glucan was visualized with Congo Red (BDH Chemicals Ltd., Toronto, ON) which does not react with degraded ß-glucan. Ground barley samples (2g) were extracted with 4 ml .1M NaCl at 4° for 1 h with frequent mixing, centrifuged (20,000g), and 50 ul of the supernatant pipetted into wells. For

very high viscosity barleys it was necessary to reduce sample size to 1 g to facilitate centrifugation. Enzyme units were expressed relative to a sample of 'Triumph' barley for which a standard curve was constructed by manipulating dilution rate.

Statistical analysis

Statistical analysis were computed suing the Statistical Analysis System (General Linear Models, Barr et al., 1979).

RESULTS

Broiler chicks given diets in which low viscosity barleys constituted the cereal base (Triumph, Nirasaki, Klages, or Riso; Experiment 1) were heavier ($P<.01$) and had lower feed conversion ($P<.01$) than chicks given high viscosity barleys (Minerva, Vada, Lami, or Yugoslavian; Table 1). Similar results were evident in Experiment 2 (Table 1), again chicks given low viscosity barleys (Triumph or Nirasaki) surpassed those given high viscosity barleys (Minerva or Yugoslavian) with respect to body weight ($P<.01$) and feed conversion ($P<.01$).

An enzyme supplement added to the diet (Table 1, Experiment 2) significantly increased body weight ($P<.01$) and lowered feed conversion ($P<.01$) for chicks fed high or low viscosity barleys. An interaction was apparent between viscosity type and enzyme supplementation for body weight ($P<.01$), but not feed conversion. While the response to enzyme supplementation was most pronounced for high viscosity barleys, chicks fed low viscosity barleys also responded substantially to enzyme treatment.

The differences observed between barleys varying in viscosity could not be accounted for by gross nutritive differences (Table 3), as most indices of nutritive value (crude protein, ADF, and ether extract) favored the high viscosity barleys. A noteworthy example was the Riso barley fed in Experiment

1 which had a high hull content yet still surpassed Yugoslavian, a hull-less cultivar, in ability to support chick growth.

The ranking of barley varieties according to viscosity for combined locations (Winnipeg, Brandon, Saskatoon, Lethbridge, and Beaverlodge) illustrated that the varieties chosen as representative of the high viscosity trait were consistently higher than those chosen for low viscosity (fig. 1a). Endogenous ß-glucanase (fig. 1b) was to closely related to viscosity, although there was a notable tendency that highest enzyme levels were found among the low viscosity barleys. Protein (fig. 1c) was not related to viscosity type.

Further examination of the impact of geographic location was conducted by dividing the barley varieties into two groups of nine, reflecting high viscosity (Colosses, Colosses IAC, Vada, Scout, Wong, Lami, Yugoslavian, and Minerva), and low viscosity (B2018, Klages, Harrington, Argyle, Nordal, SV70/2238, Triumph, Nirasaki, M737, and Riso) traits. Both viscosity type and location affected viscosity (fig. 2a); high viscosity barleys were more viscous than the low viscosity groupings at all locations. Of greater interest was the interaction; low viscosity barleys did not change appreciably among locations whereas high viscosity barleys did. The difference between the two types was greatest at Saskatoon, Winnipeg, or Lethbridge, less at Brandon, and only minor when grown at Beaverlodge. ß-glucanase levels were consistently lower for high viscosity as compared to low viscosity barleys. There were also location differences, although these did not correspond to location differences observed for viscosity. Protein levels did not difference according to viscosity type, however, location effect were apparent.

DISCUSSION

Viscosity of an acid buffer extract of barley (Greenberg and Whitmore, 1974) is reflective of soluble ß-glucan content, and may be used as a screening method to detect low total ß-glucan content (Greenberg, 1974; Aastrup, 1979). ß-glucan levels are affected by genotype (Lance, 1984), as well as environmental conditions that are conducive to moisture stress during kernel filling and ripening (Margan and Riggs, 1981; Smart, 1976). Greenberg (1977) reported a significant genotype x environment interaction for extract viscosity in a genetic study involving six barley cultivars grown at two locations, although others have found these effects were largely additive (Bourne et al., 1976).

Differences noted among acid extract viscosities of barleys examined in the present experiment reflect both genotype and environmental effects. Noteworthy were the very low viscosity values of M737 and Riso, which has been reported previously (Aastrup, 1983; Coon et al., 1979). Cultivar differences were modified by location, with those grown in the most northerly location (Beaverlodge, Alberta) exhibiting lowest viscosity. This response was confined to the high viscosity barley grouping, indicating a differential location response according to viscosity type. Extract viscosity increases logarthmically with soluble ß-glucan concentration (Greenberg, 1971), which may partly explain the response seen. Maturation of barley under warmer, and dryer conditions induced by early seeding time, or early maturation, has also been found to raise viscosity values (Classen et al., 1987). Stage of maturation at harvest also plays a role, harvesting at an 'early yellow' stage of ripeness yields higher viscosity than 'combine ripeness' (Hesselman and Aman, 1986; Hesselman and Thomke, 1982).

Endogenous ß-glucanase also varied with cultivar and location. ß-glucanase tended to vary inversely with viscosity, suggesting that lowered feeding value of high viscosity barleys may be compounded by lower enzyme levels. While increased ß-glucanase is normally related to sprouting, a second pool exists in barley associated with the husk and pericarp (Ballance et al., 1976) which may be microbial in origin (Hoy et al., 1981). Low extract viscosity would not be expected to be directly due to limited hydrolytic activity in intact grain due to physical separation of the superficial enzyme and the endospermal substrate. It has been argued that ß-glucanase, and not viscosity per se, may be the principal factor governing feeding value of barley for chicks (Burnett, 1966; Willingham et al., 1960).

Location differences were apparent for ß-glucanase levels which did not correspond to those observed for extract viscosity. Short-term weather conditions prior to and during harvest may increase enzyme levels with little effect on viscosity, which may be more responsive to conditions throughout ripening.

Protein is also related to drought stress in that incomplete kernel filling causes a relative increase in protein content. Some correspondence with extract viscosity was seen, although the relationship was weak. The barley samples in general had high protein content, possibly due to high soil nitrogen in the test plots.

The conclusion that barley extract viscosity, hence ß-glucan, bears any relevance to nutritive value of barley in drawn largely from the well-established response to dietary ß-glucanase (Classen et al., 1985; Gohl et al., 1978; Hesselman et al., 1982; Hesselman and Aman, 1986). Viscosity as a determinant of feeding value has also been noted where barley was acquired from different locations (Burnett, 1966; Willingham et al., 1960) or harvested

at different times (Hesselman and Aman, 1986).

Despite this rather convincing evidence, varietal differences in extract viscosity have not correlated to chick growth. Coon et al. (1979) observed viscosity correlated poorly to chick weight gain, although gain was poorest among the high viscosity barleys. Two barleys (Steptoe and Riso) were obvious discrepancies in the chick performance was poor despite low extract viscosity. Similarly, Gohl and Thomke (1976) found no correlation between viscosity and nutritive value of barleys grown in Sweden and concluded that tannins may be more significant.

CONCLUSIONS AND IMPACT

The results of the present experiment showed that cultivar differences with respect to extract viscosity was an important determinant of feeding value. Broiler chicks fed low viscosity barleys consistently outperformed those given high viscosity barleys. Differences in feeding value between the two barley types were eliminated with dietary enzyme supplementation. Chicks given low viscosity barleys also responded to enzyme supplementation, which, while not as substantial as that seen with high viscosity barley, was sufficient to warrant enzyme addition to all chick diets containing barley.

Given that chicks fed high extract viscosity barleys were equivalent to those given low viscosity barleys with respect to growth and feed conversion when dietary enzyme was included, any anticipated improvement by genetically lowering ß-glucan, based on the results, would be small. Lower ß-glucan could, however, reduce the quantity of enzyme added to the diet, the cost of which must be borne by the barley component of the diet alone. Also, the number of comparisons of barley diets with supplementary enzyme was small, and

factors unique to these samples may have confounded the results. It seems likely that lower ß-glucan would improve feed conversion since it, like cellulose, would be indigestible. This too has been disputed with recent reports of moderate digestibility in swine (Graham et al., 1986).

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Table 1 Performance of Broiler Chicks Fed High or Low Viscosity Barleys.[1]

	Experiment 1			Experiment 2	
Low Viscosity	Body Wt. (g)	Feed Conversion		Body Wt. (g)	Feed Conversion
Triumph	404	1.61	Triumph	449	1.56
Nirasaki	387	1.57	+enzyme	506	1.58
Klages	391	1.66	Nirasaki	435	1.63
Riso	343	1.70	+enzyme	525	1.50
High Viscosity					
Minerva	247	1.91	Minerva	347	1.79
Yugoslavian	255	1.82	+enzyme	542	1.53
Vada	255	1.94	Yugoslavian	339	1.84
Lami	316	1.72	+enzyme	519	1.55
SEM	±13	±.04		±13	±.04

[1] Body weight and feed conversion were significantly poorer for chicks fed the high viscosity barleys. Enzyme addition significantly improved both body weight and feed conversion.

FIGURE 1 EFFECT OF BARLEY VARIETY ON EXTRACT VISCOSITY, B–GLUCANASE AND CRUDE PROTEIN LEVELS.

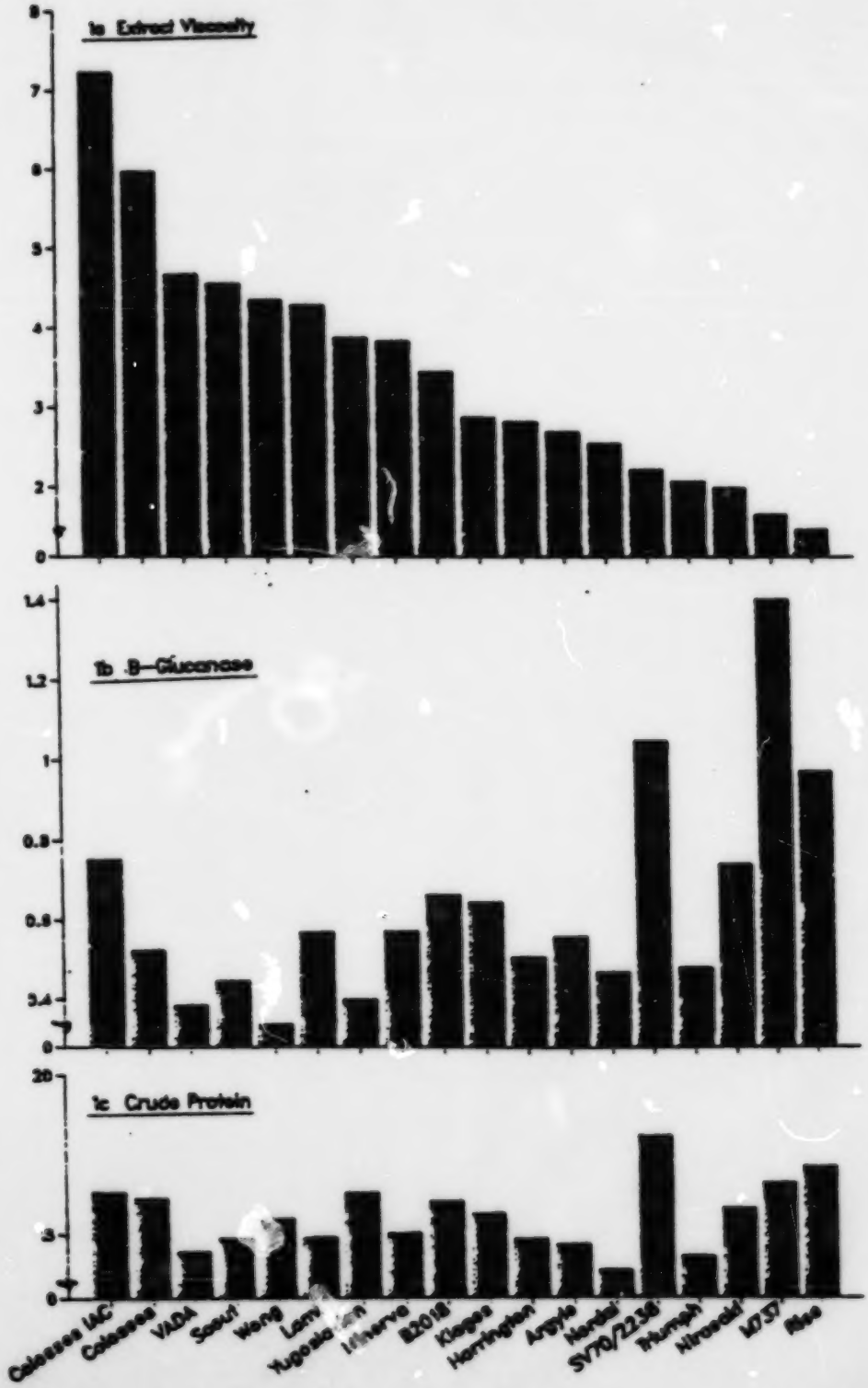
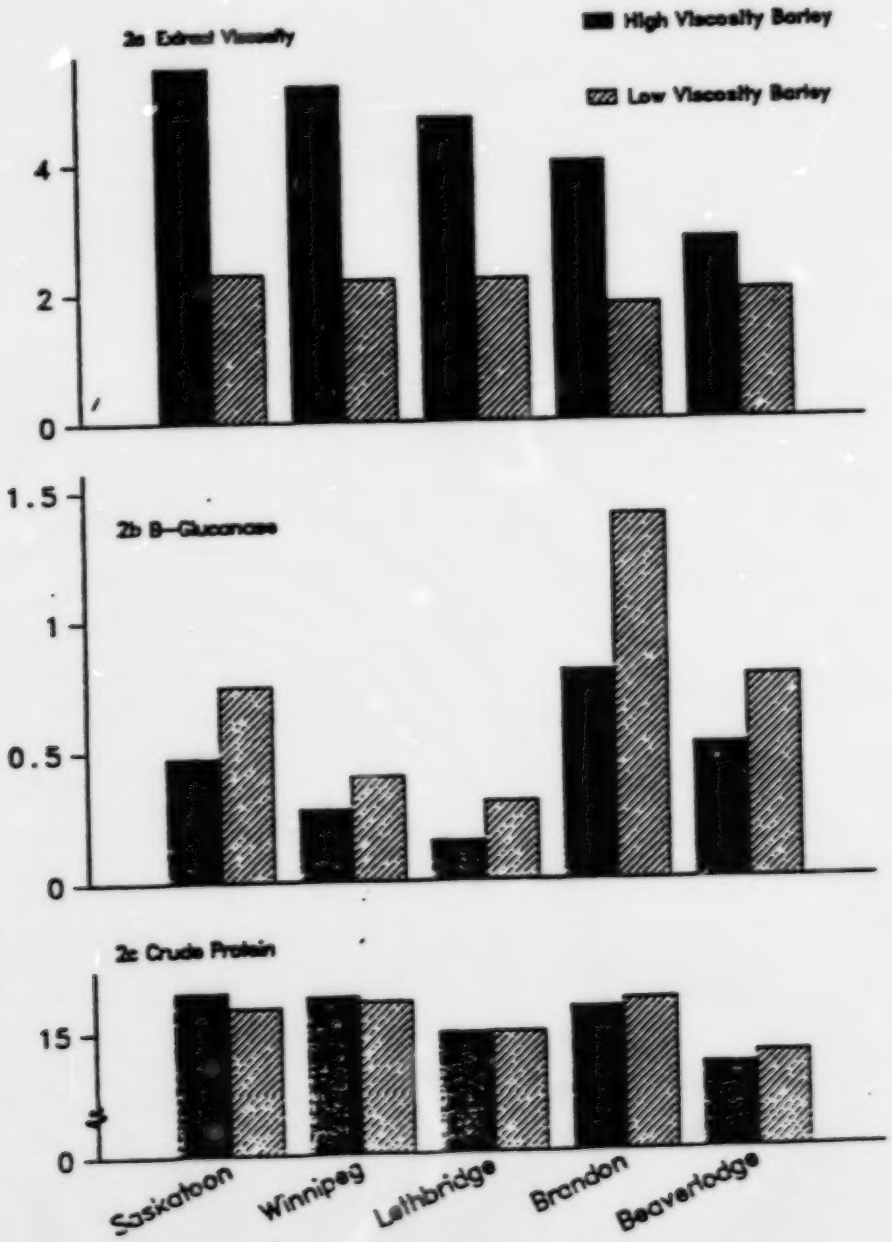


FIGURE 2 EFFECT OF GEOGRAPHIC LOCATION ON EXTRACT VISCOSITY, B–GLUCANASE AND CRUDE PROTEIN LEVELS.



c) List of papers prepared and talks given (1986):

Refereed Journals

Papers published:

Campbell, G.L., H.L. Classen, and G.M. Ballance, 1986. Gamma irradiation treatment of cereal grains for chick diets. J. Nutr. 116:560-569.

Campbell, G.L., R.E. Salmon, and H.L. Classen, 1986. Prediction of metabolizable energy of broiler diets from chemical analysis. Poultry Sci. 65:2126-2134.

Papers in press:

Campbell, G.L., F.W. Sosulski, H.L. Classen, and G.M. Ballance, 1987. Nutritive value of irradiated and ß-glucanase-treated wild oat groats (Avena fatua L.) for broiler chickens. Anim. Feed Sci. Technol.

Edney, M.J., G.L. Campbell, H.L. Classen, and R.D. Reichert, 1987. Enzyme supplementation of diets containing barley, hulless barley, or oat groats. Poultry Sci.

Papers in preparation:

Cultivar and environmental differences in extract viscosity of barley and its effect on the nutritive value for broiler chickens.

Improvement of the nutritional value of rye diets for broiler chickens by supplementation with purified pentosanase (arabinoxylanase).

Effect of dietary density on broiler carcass composition as influenced by age.

Department of Animal Science Research Reports (1986)

Campbell, G.L., R.E. Salmon, and H.L. Classen, 1986. Prediction of metabolizable energy of broiler diets from chemical analysis, pp 1-4.

Campbell, G.L., F.W. Sosulski, and H.L. Classen, 1986. Nutritive value of irradiated and ß-glucanase-treated wild oats (Avena fatua L.) for broiler chickens, pp 16-19.

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Edney, M.J., G.L. Campbell, H.L. Classen, and R.D. Reichert, 1987. Enzyme supplementation of broiler chicken diets containing barley or oats.

Presentations given:

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1. University of British Columbia, Vancouver, BC.
2. National Research Council, Ottawa, ON.

Classen, H.L.. Enzyme supplementation of broiler diets. Presented at Saskatchewan Producers' Marketing Board Extension Meetings at:

1. Balcarres, SK.
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Other:

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d) Statement of expenses

- see attached

e) Personnel

H.L. Classen, Professor - 20%
G.L. Campbell, Research Associate - 100%
D.A. Teitge, Graduate Student - 30%

Collaborative support of the Crop Development Center, University of Saskatchewan and the Plant Biotechnology Institute, NRC is acknowledged.

f) Equipment purchase

- nil